THE RELATION OF TEMPERATURE TO OXYGEN CONSUMPTION IN THE GOLDFISH [1]

F. E. J. FRY AND J. S. HART

University of Toronto

INTRODUCTION

In general, studies on the relation of the metabolic rate to temperature have been limited to a consideration of the standard metabolism. Standard metabolism in animals has been taken as the approximate equivalent to basal metabolism in man. A distinction is made in deference to the fact that animals cannot approach the experiment in such a consciously basal state as can the human subject. However, from the ecological point of view, which recognizes that much of the measure of success which an animal may enjoy is due to its ability to be active, it is not enough to know the basal or standard metabolic state alone. It is of equal importance to measure the maximum metabolic rate, unless changes in the maximum rate follow no rule, or can be predicted from the standard rate. For fish at least there appear to be no data which establish either of these two provisos. For these reasons we undertook to explore the relation between a standard and a maximum level of metabolism in the goldfish and the ambient temperature. The two levels chosen were (1) a standard which was the minimum resting level in the diurnal cycle, a level as near basal as we could establish, and (2) a "maximum" which was the rate which appeared to be the highest the fish were able to maintain continuously until fatigue set in.

MATERIAL AND METHODS

The goldfish used were a single lot of animals approaching one year old with an average weight of 3.8 gms. These fish were similar in age and source to those used for the exploration of various other physiological characteristics of the species (Fry, Brett and Clawson, 1942; Brett, 1946; Fry, Black and Black, 1947; and Fry and Hart, 1947). They were stored in running water and fed on a commercial fox food. Previous to any experiment the fish were acclimated to the particular temperature at which the experiment was to be carried out by maintaining them at that temperature for a length of time that would enable them to come to a stable state, as far as this is indicated by the stability of the upper lethal temperature (Brett, 1946). This period ranged from twenty days at 5° C. to less than four at 35° C. During this acclimation period the fish fed well. Both the standard and maximum levels of metabolism for any given temperature were measured on the same subjects, but the measurements at different temperatures were carried out on different samples drawn from the common stock. The metabolic rates were measured by determining the rate of oxygen consumption.

[1] An abstract of this paper appeared under the same title in the *Anatonomical Record*, Vol. 96, No. 4 Supp., December, 1946.

All the rates of oxygen uptake were determined by means of the unmodified Winkler method. While nitrite contamination may introduce error into oxygen determinations (Allee and Oesting, 1934) it does not seem to have been a serious factor here. A number of checks with the Rideal-Stewart modification gave essentially the same readings as the unmodified Winkler's. Moreover, consistent results were obtained on check runs in which the initial levels of oxygen differed and hence similar levels were reached after different intervals of time.

Both the standard and the maximum levels of oxygen consumption were measured by determining the fall in oxygen content in closed containers (Fig. 1). The respiration chamber used to determine the standard level was a two-liter Erlenmeyer flask. The flask was fitted with a two-hole stopper through which projected
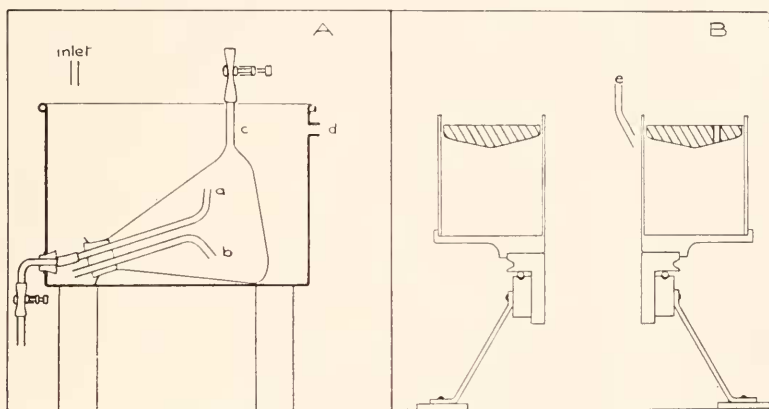

FIGURE 1. Arrangement of apparatus for measuring: A, standard metabolism; B, active metabolism. See text for detailed descriptions.

two glass tubes (a and b), the inner ends of which were bent at right angles and spread well apart. A glass side arm (c) was blown into the Erlenmeyer at is widest diameter. This side arm was provided with a length of rubber tubing and a pinch cock. The fish were placed in water in the flask which was then placed on its side in a water bath with the glass tube uppermost. Tube (a) was connected to a second tube which led through the wall of the bath. Tube (b) was open to the bath. A supply of water of the desired temperature was led into the bath and aerated vigrously. Excess water passed out an overflow (d), thus maintaining a constant level of water in the bath. Water from the bath passed by tube (b) through the Erlenmeyer as long as (a) was open. The side arm (c) collected any bubbles which formed in the flask. Normally, tube (a) was left open, and the flow through the Erlenmeyer was so adjusted as to be sufficient to make the difference in oxygen content in the inflowing and outflowing water negligible.

The chambers for measuring standard metabolism were set up in a basement room lighted by overhead lights in the daytime but dark at night, except for a shrouded fifty-watt bulb near the floor. The fish were in almost complete darkness at night since the metal bath shielded them from the light on the floor.

At temperatures of 20° C. and lower, twenty fish were placed in the Erlenmeyer; at higher temperatures the number was reduced to ten. The fish were placed in

the chambers on the day previous to the experiment, and water samples for oxygen determination were taken as described below over at least one complete twenty-four hour cycle. All samples were taken as quietly as possible in order to minimize any disturbance of the subjects. However, at that time we were not conversant with the findings of Spoor (1946), and we undoubtedly did not eliminate such disturbance entirely. The minimum oxygen consumption generally occurred between 10 p.m. and dawn. The titration figures for each twenty-four hour cycle were plotted, and the lowest point during the night hours found by interpolation. The standard rate of oxygen consumption was calculated from the value thus found.

When a measurement of the oxygen consumption was to be made, any bubbles in tube (c) were carefully drawn out by suction. Water samples were taken from tube (a) and the bath. Tube (a) was then closed by the screw cock, thus isolating the water in the flask except for the connection through the relatively long and narrow tube (b). The time interval between the first and second sample was dependent on the metabolic rate. These intervals varied from four hours at 5° C. to thirty minutes at 35° C. The time between the initial and final samples was so chosen that the oxygen tension in the chamber did not fall low enough to affect the rate of oxygen uptake, a number of preliminary experiments being performed to confirm this fact. After the appropriate interval of time a second sample of approximately 100 cc. was drawn from tube (a) into a 50 cc. sample bottle, the first 50 cc. being used to flush, the second 50 cc. being retained as the sample. This partitioning of the sample was accomplished by taking the time during the first filling of the bottle and then allowing the water to flow for a further equal time before removing the sample bottle. It was presumed that this relatively rapid withdrawal of 100 cc. took place without any contamination from the corresponding 100 cc. of water which entered the Erlenmeyer from the bath.

The maximum level of metabolism was measured on fish swimming steadily against a current generated in a rotating chamber (Fig. 1B). This chamber was an annular vessel of rectangular cross section of which the outer wall was glass and the inner wall and bottom metal. The outside diameter was 12 inches, the width 3 inches, and the depth 6 inches. This apparatus is an elaboration of that originally used by Black, Fry and Scott (1939). A measured volume of water was placed in the chamber, the fish introduced, and the surface of the water protected from the atmosphere by means of a loosely fitting cover. Samples of water for the determination of the oxygen content were obtained by withdrawing 100 cc. by pipette. The pipette was then inserted to the bottom of a 50 cc. sample bottle and approximately 75 cc. of the sample was delivered into it, the excess being flushed out. The last 25 cc. in the pipette were discarded. Since the cover did not isolate the water completely from the atmosphere, some exchange of gas took place between the water and the air. The magnitude of this exchange at different temperatures and oxygen tensions was determined by filling the chamber with water which had been boiled and flushed with nitrogen, and then following the increase in oxygen with the cover in place. We are indebted to Miss J. M. Graham for making these determinations. A correction based on these results was applied to the apparent rates of oxygen uptake determined experimentally. The temperature of the water in the rotating chamber was controlled by playing a stream of water on its inner wall through tube (e).

Results

(a) *Relation of temperature to standard metabolism*

The relation of the standard metabolism of the goldfish to temperature has been investigated by other workers on a number of occasions. On two occasions in particular (Ege and Krogh, 1914; Gardner, King and Powers, 1922) this relation has been worked out over a considerable range of temperature. The results of these workers are displayed with our own in Figure 2. The logarithms of the
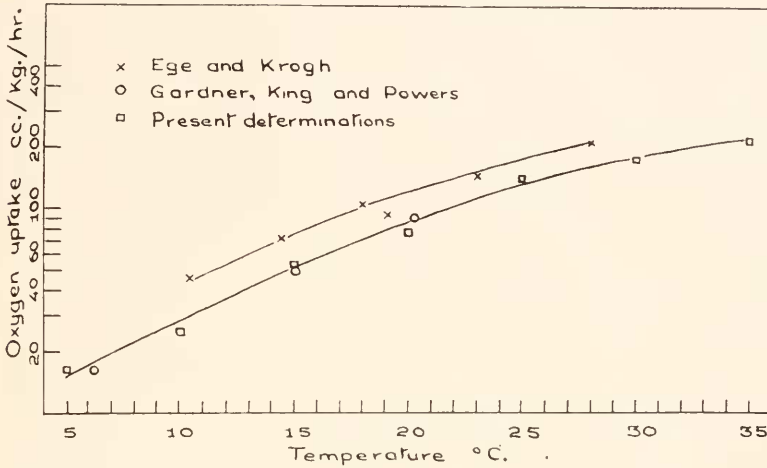

FIGURE 2. The relation of temperature to standard metabolism in the goldfish.

rates of oxygen uptake are used in this figure rather than the actual values so that a comparison can be made between the relative rates of change. The relative rates of change with increase in temperature are very similar in the three sets of data. Since different techniques were used in the three cases, such agreement can probably be taken as a satisfactory indication that the relative change is a stable characteristic of the species, although it must be remembered that this particular curve is characteristic of quite a number of other animals also (Krogh, 1941).

There does not appear to be any indication of any difference in this relative rate with difference in size such as wells (1935) believed was the case in *Fundulus parvipinnis*. However, his evidence is somewhat confused by the fact that the rates at different temperatures were measured at different times of day. Moreover, a good deal of the divergence in the rate-temperature curves he gives is an attribute of the magnitude of the absolute rates and disappears if a semi-logarithmic plot is used, as in Figure 2.

The rate of oxygen uptake per unit weight does not bear any consistent relation with size in the three sets of data in Figure 2. Such disagreement is probably due to the fact that the standard states used by the three groups of investigators were not the same.

(b) Relation of temperature and oxygen tension to the maximum rate of oxygen uptake

So far as we are aware, no other workers have explored the effect of temperature on the maximum rate of oxygen uptake by the goldfish, nor have we examined this at all exhaustively, for we have confined our observations to what we believe to be the maximum level of oxygen uptake that can be maintained steadily.

The maximum rate was measured over a range of oxygen tensions down to those approaching asphyxial levels. In this way the effects of oxygen and temperature can be determined simultaneously and the scope of the experiment thus extended. The technique used was the familiar and simple one of allowing the organism to exhaust the oxygen in an isolated volume of water. In the case of goldfish it can probably be safely assumed that the small increase in carbon dioxide that results from the respiratory exchange in such experiments has no appreciable depressing effect on the rate of oxygen uptake. Under the conditions of our experiments the highest tension this respiratory carbon dioxide would reach in Toronto tap water is about 20 mm. at 35° C., a level which would appear to be far from any that would exert a limiting effect (Fry, Black and Black, 1947).
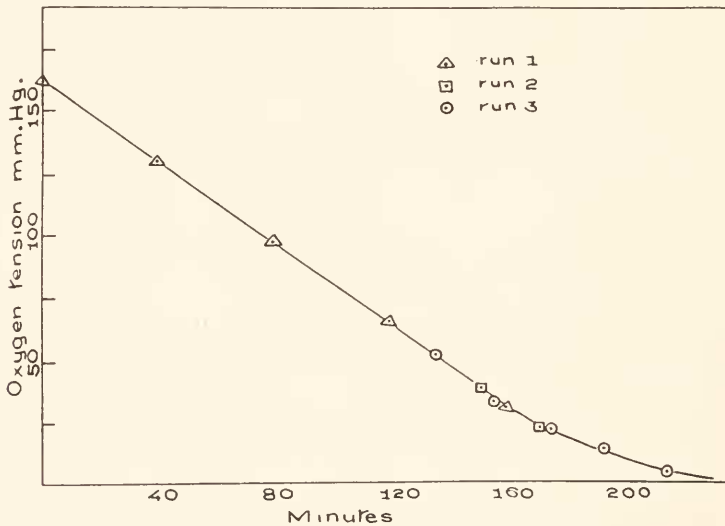

FIGURE 3. The time course of oxygen depletion in the rotating chamber, in a typical measurement of the metabolism of active goldfish (temperature 15° C., 20 fish, 4 litres water).

The course of the removal of oxygen from the water in the respiration chamber in a typical experiment is shown in Figure 3. Figure 3 presents the same picture as that found by Toryu (1927) for this species. Thus, our figures confirm his findings that the gold fish, at least when of the size of our subjects, has a respiratory system that is independent of the oxygen tension down to relatively low levels even under conditions of maximum continuous activity.

The maximum steady rates of oxygen uptake in relation to oxygen tension found at the different temperatures investigated are shown in Figure 4, where the rate of

oxygen uptake is plotted gainst oxygen tension. These curves were derived by taking tangents from the appropriate graphs similar to Figure 3. Owing to the low level at which the oxygen uptake becomes dependent on oxygen tension, the data on which the descending portions of these curves are based are rather meager and no precise accuracy is claimed for them such as was attained by Maloeuf (1937) in his investigations. In particular, the curve determined at 20° C. is not consistent with the trend of the remaining data. However, although the proportional error may be considerable, the absolute error must be of no great order since the range of tensions occupied by this portion of the curve is in all cases less than 40 mm.
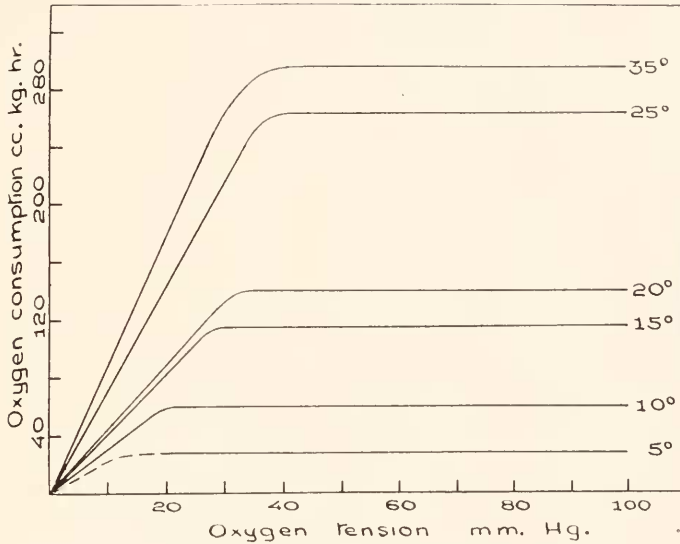

FIGURE 4. The relation of the maximum steady rate of oxygen consumption to oxygen tension at various temperatures.

While the physiological significance of the phenomenon commonly known as respiratory dependence has been often discussed at length since the beginning of the century, its ecological implications do not appear to ever have been clearly stated, except in connection with man in the case of the conquest of Mount Everest (Henderson, 1939).

The data in Figures 2 and 4 appear to us to offer two physiological indices that may be of great use to the ecologist. These have been designated by Fry (1947) as "the incipient limiting level" and "the level of no excess activity." The incipient limiting level is that level of oxygen tension below which the rate of oxygen uptake at the maximum steady state of activity begins to be reduced. This is the point of inflection of the curves in Figure 4. The incipient limiting levels of oxygen tension thus determined for the goldfish are given in Table I. The point of inflection in Toryu's data occurs at an oxygen tension of the order of these values, which appear to us to indicate that the respiratory rate he was measuring was well above the standard level under the conditions to which he was subjecting his animals. Such circumstances have been commonly remarked.

The level of no excess activity has been taken as that level of oxygen at which the animal can no longer satisfy more than its standard requirements for oxygen in spite of the utmost efforts of its ventilatory and circulatory apparatus. This level for the goldfish at a given temperature can be determined by finding on the curves in Figure 4 the tension corresponding to the oxygen consumption under standard conditions at the same temperature as determined from Figure 2. These values are presented in Table II.

TABLE I

Tensions of oxygen at various temperatures below which the maximum steady rate of oxygen uptake is reduced in young goldfish acclimated to the temperatures at which the measurements are made

Temperature °C.	Incipient limiting level of oxygen mm. Hg
5	15
10	21
15	29
20	34
25	39
35	40

TABLE II

Estimated oxygen tensions at which young goldfish can take up no more oxygen than will satisfy the requirements of standard metabolism in subjects acclimated to the temperature at which the estimate is made

The values for the standard metabolic rate in column 1 were taken from the smooth curve through our data in Figure 2.

Temperature °C.	Standard met. rate cc. Kg. hr.	Level of no excess activity mm. Hg	Asphyxial level[1] mm. Hg	Residual level[2] mm. Hg
1				4
2.6			nil	
5	8	4		
7				4
10	24	8		
11.4			8	
15	50	10		4
20	85	18		7
25	140	17		8
27.2			12	
32				8
35	225	25		

[1] Gardner and King, 1922.
[2] Fry, Black and Black, 1947.

The levels of no excess activity given in Table II have, of course, been abstracted from a purely experimental situation, and the worth of such data to the ecologist must ultimately depend on proof that they have real significance as values limiting the activity of the organism in nature. The greatest uncertainty lies in the assumption that the standard level chosen was a fair approximation of the minimal

respiratory requirements of the animal. We have not investigated the matter exhaustively, but a comparison is made in Table II with the values given by Gardner and King (1922) for the asphyxial level of oxygen for goldfish and what for convenience have been termed "residual levels" given by Fry, Black and Black (1947). These residual levels are the tensions of oxygen which remain in water in sealed bottles in which the fish have been allowed to exhaust the oxygen supply until they died. Thus, since some oxygen transport still goes on after the animals have been asphyxiated, and since the volume of water in which the fish were confined was small these residual values are somewhat below the asphyxial level. Taking the various circumstances into consideration, these three sets of data show fair agreement.
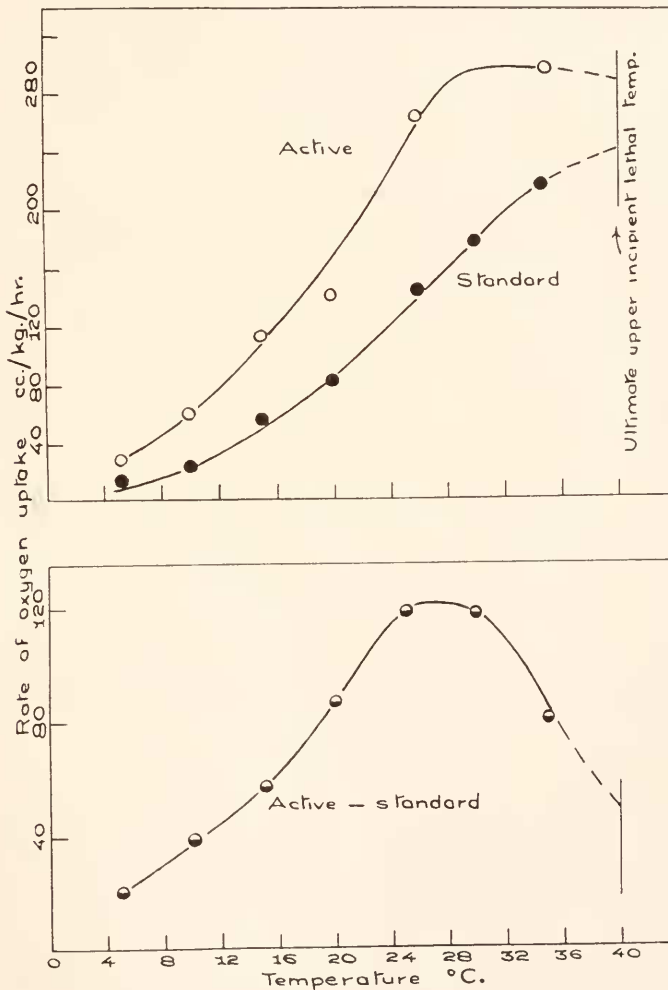

FIGURE 5. The relation between temperature and the active and standard levels of oxygen uptake.

(c) The difference between the maximum and standard levels of metabolism at various temperatures

Assuming that the standard level of metabolism as measured here is a reasonable approximation of the maintenance metabolism, then the metabolism available for activity will be of the order of the difference between the maximum and standard levels. It will not be the whole difference, since with increasing activity the cost of ventilation and circulation and the other functions necessary to allow the external work to be carried on will, of course, increase, and some of the difference will be consumed for the increase of these auxiliary activities. The relation between temperature and the maximum rate of oxygen uptake at levels where the oxygen tension is not limiting, that is, at levels along the horizontal portions of the curves in Figure 4, is shown, together with the curve for standard metabolism in Figure 5.
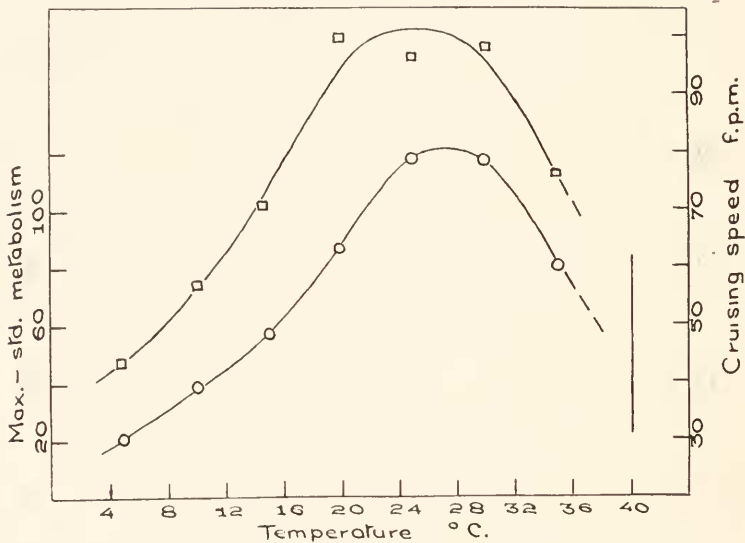

FIGURE 6. A comparison of the difference between the maximum and standard levels of oxygen uptake (lower curve) and the speed at which goldfish can swim steadily at various temperatures. The vertical line at 40° C. indicates the ultimate upper incipient lethal temperature of the goldfish.

In Figure 5 it will be seen that while the curve of standard metabolism probably increases with increasing temperature right up to the ultimate incipient lethal temperature (Fry, Brett and Clawson, 1942; Fry, Hart and Walker, 1946), the same is not true of the curve of maximum oxygen uptake. This latter curve reaches a height at about 30° C. which apparently represents the maximum capacity of the system involved, for it is not surpassed at higher temperatures. The consequence of the difference in course of the two curves representing the maximum and standard rates of oxygen uptake is that the difference between them reaches a maximum in the neighborhood of 28° C. and falls off rapidly above 30° C. The difference between the maximum and standard rates is given in the lower panel of Figure 5.

If the difference between the maximum and standard rates of metabolism does approximate the metabolism available for external work, then it would appear that there is an optimum temperature in the neighborhood of 28° C. at which goldfish can perform the most external work. This deduction from the data on metabolism is supported by observations on the relation of temperature to cruising speed (Fry and Hart, 1947). The curve illustrating the relation between temperature and the speed at which goldfish can swim steadily also appears to show a maximum between 20° and 30° C., although somewhat flatter than that for the difference between maximum and standard metabolism. The curves for cruising speed and the metabolic difference are compared in Figure 6. Thus, there seems to be the same correlation between oxygen consumption and activity over the whole temperature range that Spoor (1946) demonstrated at 23–25° C.

DISCUSSION

The data presented on the maximum and standard rates of oxygen uptake point to an explanation of activity-temperature curves which drop at the upper end of the biokinetic range, which is alternative to the theory of thermal destruction usually invoked in such cases. The standard metabolism appears to increase with increasing temperature right up to the ultimate incipient lethal temperature. There is certainly no evidence of destruction or impairment of respiratory enzymes concerned with the standard metabolic rate up to a temperature of 35° C. On the other hand, the curve for maximum metabolism would appear to level off above 30° C. Such levelling off might be caused by a destruction of enzymes at higher temperatures to a degree which produces a balance between the effects of the increased temperature and the ability of the organism to respond, but some proof of this assumption is necessary if it is going to be offered as the explanation. Moreover, to explain the drop in cruising speed above 30° C. on the basis of thermal destruction of enzymes would then require that the explanation be applied to the maximum metabolic rate alone. However, a simpler explanation would be that the levelling of the maximum rate is because the capacity of some part of the organism concerned in oxygen transport is reached at about 30° C. However, whatever may be the explanation of the course of the curve of maximum metabolism, the correlation between activity and metabolism is not with either of the metabolic levels investigated here but with the difference between them. Or more properly, it is with some function of the difference between the two curves which ideally measure the metabolic levels and which our measurements approximate as is indicated by the correlation shown in Figure 6.

With respect to technique it appears that the simplest and most direct methods of obtaining ecologically significant values of oxygen uptake are to measure the maximum rate over a series of varying oxygen tensions down to the asphyxial level, and to measure the standard rate over a range of tensions at which oxygen would not be the limiting factor even for the maximum rate. In the case of the gold fish, for reasons stated above, carbon dioxide tension has not been considered, but for many other species it cannot be so ignored. In many respects however, there seems to be no major objection to combining the effects of carbon dioxide increase and oxygen decrease since they are combined in nature, particularly if the experiments are performed in water low in dissolved minerals so that a maximum increase in carbon dioxide tension will be attained for a given decrease in oxygen. Thus the limiting

values for oxygen attained will approach the maximum that would be expected to be found in nature.

SUMMARY

1. Two levels of oxygen uptake, (1) the lowest point in the resting metabolism in the daily cycle and (2) the maximum steady rate of oxygen uptake found when the fish were stimulated to activity in a rotating chamber, were measured at temperatures from 5° to 35° C.

2. The standard (resting) rate was measured over levels of oxygen high enough to avoid any dependence of the rate on oxygen tension. The maximum rate was measured over a series of oxygen tensions down to the asphyxial level.

3. The standard rate continued to increase with temperature up to 35° C., the highest temperature at which observations were made. The maximum rate was found by interpolation to reach its highest value at about 30° C. and to remain steady or decrease slightly at higher temperatures.

4. The maximum rate of oxygen uptake became dependent upon the oxygen tension between 15 and 40 mm. Hg, depending upon the temperature. These estimates were made in a closed system in which there was an accumulation of the carbon dioxide released in respiration.

5. Oxygen tensions at which the maximum oxygen uptake met only the needs of the standard metabolism were estimated to be between 4 and 25 mm. Hg over the temperature range investigated.

6. At the various temperatures the difference between the maximum and standard metabolic rates is correlated with the rate at which goldfish can swim steadily.

7. It is concluded that the drop in the sustained swimming rate of goldfish at temperatures from 30° to 38° C. is probably due to a decrease in the metabolism available for external work rather than to the thermal destruction of enzymes.

LITERATURE CITED

ALLEE, W. C., AND R. OESTING, 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiol. Zool.,* **7**: 509–541.

BLACK, E. C., F. E. J. FRY, AND W. J. SCOTT, 1939. Maximum rates of oxygen transport for certain freshwater fish. *Anat. Rec.,* **75**: Supp. 80.

BRETT, J. R., 1946. Rate of gain of heat tolerance in goldfish. *Univ. Toronto Stud. Biol. Series 53. Pub. Ont. Fish. Res. Lab.,* **64**: 21 pp.

EGE, R., AND A. KROGH, 1914. On the relation between the temperature and respiratory exchange in fishes. *Int. Rev. Hydrobiol. and Hydrograph.,* **7**: 48–55.

FRY, F. E. J., 1947. Effects of the environment on animal activity. *Univ. Toronto Stud. Biol. Series 55. Pub. Ont. Fish. Res. Lab.,* **68**: 62 pp.

FRY, F. E. J., AND J. S. HART, 1947. The cruising speed of goldfish in relation to water temperature. *Jr. Fish. Res. Bd. Canada,* 7 (4). In press.

FRY, F. E. J., V. S. BLACK, AND E. C. BLACK, 1947. Influence of temperature on the asphyxiation of young goldfish (*Carassius auratus* L.) under various tensions of oxygen and carbon dioxide. *Biol. Bull.,* **92**: 217–224.

FRY, F. E. J., J. R. BRETT, AND G. H. CLAWSON, 1942. Lethal limits of temperature for young goldfish. *Rev. Can. de Biol.,* **1**: 50–56.

FRY, F. E. J., J. S. HART, AND K. F. WALKER, 1946. Lethal temperature relations for a sample of young speckled trout (Salvelinus fontinalis). *Univ. Toronto Stud. Biol. Ser. 54. Pub. Ont. Fish. Res. Lab.,* **66**: 5–35.

GARDNER, J. A., AND G. KING, 1922. Respiratory exchange in freshwater fish, Part IV. Further comparison of goldfish and trout. *Biochem. Jour.,* **16**: 729–735.

GARDNER, J. A., G. KING, AND E. B. POWERS, 1922. The respiratory exchange in freshwater fish. III. Goldfish. *Biochem. Jour.,* **16**: 523–529.

HENDERSON, Y., 1939. The last thousand feet on Everest. *Nature,* **143**: 921–923.

KROGH, A., 1941. *The comparative physiology of respiratory mechanisms.* Univ. of Penn. Press, Phila.

MALOEUF, N. S. R., 1937. Studies on the respiration of animals, I and II. *Zeit. f. vergl. Physiol.,* **25**: 1–28, 29–42.

SPOOR, W. A., 1946. A quantitative study of the relationship between the activity and oxygen consumption of the goldfish, and its application to the measurement of respiratory metabolism in fishes. *Biol. Bull.,* **91**: 312–325.

TORYU, Y., 1927. Respiratory exchange in Carassius auratus and the gaseous exchange of the air bladder. *Sci. Rept. Tohoku Imp. Univ. L.,* **3**: 87–96.

WELLS, N. A., 1935. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. *Physiol. Zool.,* **8**: 196–227.